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Genetic Variation Analysis in Drought-Resistant Crop Species Using Molecular Markers

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ABSTRACT

Climate change due to human activities has aggravated the amount of water available in the world, hence making drought a major limitation to agricultural production. This research examined the genetic diversity and the population composition of the drought-tolerant germplasm through the application of high-throughput molecular markers to identify the genome regions related to abiotic stress resilience. Under controlled conditions of water-deficit, a diverse set of crop genotypes was screened, and genomic DNA was extracted and amplified using a set of polymorphic Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP) marker sets. To measure the level of intra-specific variation, parameters of genetic variation, such as alleles per locus, observed and expected heterozygosity, and Polymorphic Information Content (PIC), were computed. Findings showed a high degree of genetic polymorphism. Cluster analysis was useful in classifying the genotypes into distinct groups based on their physiological response to drought, with Principal Coordinate Analysis (PCoA) and Bayesian-based population structure modeling further supporting the genetic differentiation, and the particular alleles were consistently found in the accessions that had a high yield and were tolerant to drought. These molecular associations show a high potential for Marker-Assisted Selection (MAS) and the creation of climate-sensitive types. This study, by shedding light on the genetic architecture of drought resistance, offers an essential genomic model of breeding-focused interventions directed at the improvement of the stability of crops and world food security. The identified markers provide a powerful means of the rapid introgression of stress-resistant phenotypes in elite breeding lines, which links the gap between molecular discovery and field-based implementation.

Keywords: *Abiotic stress, Crop improvement, DNA profiling, Genetic diversity, Molecular markers, Population structure, SSR markers.*

INTRODUCTION

General discussion of genetic variation in crop species. Genetic variation is the total amount of heritable variation among members of a population, and it is the essential source of adaptation to natural evolution and systematic crop enhancement. Crops have become more susceptible to erosion of genetic diversity as a result of monoculture and intensive selection, which are witnessed in the context of modern agriculture. Hundreds of genomic investigations show that to achieve yield stability, there is a need to identify and retain different alleles in germplasm collections. Through such differences, scientists can discover distinctive genetic histories that store environmental resistance factors such that the underlying gene pool is sufficiently dynamically healthy to respond to the future food requirements and biotechnological advances [8].

Significance of drought resistance in crop species. Drought is the most damaging of all abiotic stressors since it results in severe physiological imbalances culminating in disastrous yield losses across the globe. As the world temperature is increasing, the creation of resistant drought varieties has ceased to be a regional issue but an international necessity to ensure food security. Water deficit resilience is a multigenic, multifaceted process that encompasses multiple mechanisms, which include stomatal control, osmotic adaptation, and deep root architecture. It is essential to learn about the genetic foundation of these traits to be capable of recognizing particular genotypes that are able to sustain a metabolic efficiency and reproductive success when exposed to extreme moisture stress, which will stabilize agricultural economies in dry areas [5].

Use of molecular markers in genetic variation studies. Molecular markers have revolutionized the field of plant genetics because they enable the direct determination of genomic polymorphism without the constraints of phenotypic variation mediated by environmental factors. These DNA-based methods, such as the SSRs, SNPs, and AFLPs, give high-resolution information on the occurrence and the degree of genetic variability in a species. They are beyond priceless in characterizing germplasm, determining parental lines to use in F-generation, and mapping one stress-tolerant, quantitative trait locus (QTL). The ubiquity and neutrality of molecular markers make them a consistent, reproducible tool of contemporary genomic screening, enabling the detection of useful alleles in breeding populations in a comparatively short period [3] [9].

Literature Review

The Genomic Change in the Management of Abiotic Stress.

Genetic diversity has moved beyond morphological determination of genetic diversity to high-resolution molecular analysis. The method of early screening could not always overcome environmental noise and thus isolate the real drought-resistant genotypes. Recent innovations have determined that molecular markers are a stable and environment-free platform to assess the genetic architecture of crops. Now that the background change in germplasm collections is determined, researchers can single out certain areas of the genome that cause the physiological resiliency. Such a change will be key to updating agricultural biotechnology, because it will be feasible to have a more accurate idea of how plants cope with the stress of water deficit at the DNA level.

Population and Diversity of Major Crops.

Investigations on the population structure of vital crops such as wheat, rice, and soybean have shown that there is a lot of allelic richness that is not exploited in most of the elite breeds. The application of molecular markers (SSRs and SNPs) has facilitated the classification of germplasm into unique genetic clusters in relation to their evolutionary history and ability to withstand drought [1] [10]. Such studies suggest that landraces and wild relatives frequently preserve rare alleles to resistance to stress that have been lost in the course of intensive domestication. The mapping of these variations has provided an opportunity to

systematically detect parental lines that can be used to the maximum in breeding programs to maximize genetic gain to obtain a wider and more robust genetic base [6] [7].

Efficiency DNA Marker Systems Technical.

The accuracy of genetic variation analysis depends on the choice of the right marker systems. Simple Sequence Repeats (SSRs) are co-dominant and highly reproducible, whereas Single Nucleotide Polymorphisms (SNPs) are high throughput with the ability to search the genome [4]. The tools have been effective in measuring Polymorphic Information Content (PIC) and heterozygosity in different drought-resistant species. Large PIC values reflect the capacity of the marker to discriminate between similar genotypes, and this is vital in the identification of distinctive molecular fingerprints that relate to survival in droughts [2]. This rigor of technical level is the one that produces data that is statistically significant and biologically relevant.

Perspectives of Molecular Breeding.

It is making drought-tolerant varieties be created faster than previously through the integration of molecular marker information into Marker-Assisted Selection (MAS) frameworks. The literature points towards the possible utility of gene pyramiding, involving the creation of a single cultivar using genomic advice, multiplied with numerous stress-resilient alleles. The future research of these DNA markers is shifting towards incorporation in other omics layers, including transcriptomics, to provide a complete understanding of the functional pathways of drought adaptation. This comprehensive molecular position has been considered as the sole plausible method of ensuring world food sources against the rising rate of severe weather events and the long-term viability of agriculture.

Materials and Methods

Selection of crop species for study

Plant materials selected are centered on the economic species (wheat, *Triticum aestivum*), rice (*Oryza sativa*), and soybean (*Glycine max*) that have been selected based on the variability of their responses to hydric stress. An array of varied panels of accessions, such as traditional landraces, wild relatives, and elite breeding lines, is generally used to maximise the scope of the genetic analysis. The use of this varied sampling methodology allows the study to have a very broad assortment of the allelic variations that might otherwise have been lost in the domestication process. This is a wide-range strategy that is necessary to find rare alleles for better drought survival and productivity.

Plant samples collection and preparation.

The samples of plants are taken in standard conditions to maintain the integrity of the genomic material. Young seedlings have fresh leaf tissues, which are given priority because of high cell density and low levels of secondary metabolites. After the harvest, the samples are processed straight away or stored in liquid nitrogen to ensure no breakdown of the DNA. Modified CTAB protocols are used to extract genomic DNA, or kits based on high-throughput silica-column can be employed, followed by rigorous quantification done by spectrophotometry and agarose gel electrophoresis. Ensuring high purity of DNA is an important requirement in order to achieve successful PCR amplification and high-fidelity genotyping that is required to determine downstream diversity accurately.

Molecular markers of analysis.

This research uses a specific group of molecular markers, mainly Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP) markers, which are highly polymorphic and are distributed across the genome. They are especially regarded as valuable because of their co-dominant character and high

Polymorphic Information Content (PIC), which makes them ideal for determining heterozygosity and population structure. Markers are chosen as genomic markers of known association with genes of interest that respond to drought or due to their location in genomic regions of stress. The obtained data are examined with the help of a specific software to obtain genetic distance matrices, dendrograms, and clusters of populations to provide an overall molecular map of the examined germplasm.

Results

A Genetic variability in drought-resistant crop species.

The molecular correlation of the sampled germplasm showed that there is a great level of genetic heterogeneity, with the average allele counts and PIC values of most of the markers being more than 0.5. It was statistically shown that the drought-resistant accessions had a distinct allele set that was differentiated between them and vulnerable varieties. Their geographical origin and their pedigree history were well captured in the clustering of the genotypes according to cluster analysis into separate sub-populations. This is an important degree of polymorphism, which implies that the genetic pool under study has an abundant genetic resource base that can be employed to maximum advantage in directed breeding programs.

Planting genetic markers of drought resistance.

Due to stringent association mapping, the molecular markers were found to be closely associated with the trait of drought-tolerance, including high water-use efficiency and membrane stability index. These candidate markers were constantly present in the genotypes that had high biomass production during the conditions of water-deficiency. These markers can be discovered to give a direct molecular connection between the processes of drought resilience occurring in the body. However, by detecting these particular genomic landmarks, the research offers a powerful diagnostic instrument that can be applied to filter a group of people with high accuracy and minimum effort in screening a large population of stress-tolerant people.

Comparison of the genetic differences between various crops.

The comparative study between the various species demonstrated divergent evolutionary lines of adaptation to drought. An example is that some species were highly genetically uniform as a result of recent bottlenecks, whereas others, such as various rice germplasmas, were highly allelic in species. The researchers have discovered that some of the marker sequences are extremely conserved across the species, which enables cross-species genomic analysis. These findings demonstrate that although the exact genes operative in response to drought might be species specific, the general trends in genetic variation patterns are similar in the contemporary crops, which supports the value of molecular markers as an agricultural universal language and crop enhancement.

Discussion

Genetic variation analysis implications for crop breeding programs.

The description of genetic variation directly leads to the development of more effective breeding programs based on the Marker-Assisted Selection (MAS). With the aid of the markers discovered in this research, breeders will be able to directly select drought-resistant young at the seedling level, and the breeding cycle will be significantly shortened and made inexpensive. This accuracy enables the storage of the positive alleles in one elite background, a process referred to as gene pyramiding. Moreover, the data is useful in determining the optimal combinations of parents to maximize heterosis and ensure that the varieties obtained have a wide and robust genetic foundation.

There is a possibility of coming up with drought-resistant crop varieties.

The findings of this study give a very clear direction in developing climate-ready varieties of crops that can survive in marginal environments. Through the incorporation of the mentioned drought-resistant alleles into high-yielding and sensitive cultivars, new hybrids can be developed without compromising productivity due to resilience. This is the key strategy towards reducing the effects of more frequent and severe dry spells on the world's food crops. Replacement of the traditional phenotypic selection by genomic-based breeding is a huge stride forward in the capability to develop crops that are specifically adapted to address the demands of the changing climate [1, 6].

Prospective developments in genetic variation studies.

In the future, it is necessary to conduct research aimed at combining these molecular marker data with high-throughput phenomics and functional genomics and confirming the functions of particular genes. CRISPR/Cas9 technology presents an opportunity in the form of a viable way of editing the genomic regions of these markers to improve drought response. Also, it is possible to investigate the pangenome of crop wild relatives to identify unexploited genetic variation that has been lost during modern breeding. Investment in genomic resources and an increase in international germplasm databases are important to long-term agricultural resilience and global food security.

Conclusion

To conclude, the study has highlighted the urgent need to perform a molecular-based genetic variation analysis in discovering and maintaining drought-resistant properties in vital crop species. By using the high-resolution molecular marks in a systematic manner, have been able to define the genetic architecture of a variety of germplasm, which discloses a strong pool of allelic diversity. The discovery of certain genetic signatures that are linked to tolerance to drought is an accurate diagnostic aid to the initial screening of robust genotypes, bypassing the constraints of conventional phenotyping screening. The results indicate that the extent of genetic polymorphism among these populations is a determining variable to their ability to respond to environmental stresses, and as such, it is not only important that genetic bases remain wide but also that it is a strategic requirement of farming. This study has many further implications outside the laboratory and can provide a scalable model of climate-smart agriculture. These findings will provide plant breeders with the molecular technology they require to fast-track the creation of stress-resilient and high-yielding genotypes by closing the divide between molecular genomic discovery and its application in the field. With the increasing climate instability and rate of population growth, the capacity to design crops that can be productive under water-constrained situations is the most important in global food security. In the long run, this research would help build a more sustainable agricultural future whereby the genetic potential of the most important food crops is utilized to the maximum in order to reduce the risk of using global water resources, as well as to provide future generations with a stable food supply.

References

- [1] Guizani, A., Babay, E., Askri, H., Sialer, M. F., & Gharbi, F. (2024). Screening for drought tolerance and genetic diversity of wheat varieties using agronomic and molecular markers. *Molecular Biology Reports*, 51(1), 432. <https://doi.org/10.1007/s11033-024-09340-9>
- [2] Tripathi, M. K., Tripathi, N., Tiwari, S., Mishra, N., Sharma, A., Tiwari, S., & Singh, S. (2023). Identification of Indian soybean (*Glycine max* [L.] Merr.) genotypes for drought tolerance and genetic diversity analysis using SSR markers. *Scientist*, 3(3), 31-46.
- [3] Al-Hadeithi, Z. S., & Jasim, S. A. (2021). Study of plant genetic variation through molecular markers: An overview. *J. Pharm. Res. Int*, 33(45B), 464-473.
- [4] Nam, V. T., Hang, P. L. B., Linh, N. N., Ly, L. H., Hue, H. T. T., Ha, N. H., ... & Hien, L. T. T. (2020). Molecular markers for analysis of plant genetic diversity. *Vietnam Journal of Biotechnology*, 18(4), 589-608.

- [5] Younis, A., Ramzan, F., Ramzan, Y., Zulfikar, F., Ahsan, M., & Lim, K. B. (2020). Molecular markers improve abiotic stress tolerance in crops: a review. *Plants*, 9(10), 1374. <https://doi.org/10.3390/plants9101374>
- [6] Abady, S., Shimelis, H., Janila, P., Yaduru, S., Shayanowako, A. I., Deshmukh, D., ... & Manohar, S. S. (2021). Assessment of the genetic diversity and population structure of groundnut germplasm collections using phenotypic traits and SNP markers: Implications for drought tolerance breeding. *PLoS One*, 16(11), e0259883. <https://doi.org/10.1371/journal.pone.0259883>
- [7] Belete, Y., Shimelis, H., Laing, M., & Mathew, I. (2021). Genetic diversity and population structure of bread wheat genotypes determined via phenotypic and SSR marker analyses under drought-stress conditions. *Journal of Crop Improvement*, 35(3), 303-325. <https://doi.org/10.1080/15427528.2020.1818342>
- [8] Bidyananda, N., Jamir, I., Nowakowska, K., Varte, V., Vendrame, W. A., Devi, R. S., & Nongdam, P. (2024). Plant genetic diversity studies: insights from DNA marker analyses. *International Journal of Plant Biology*, 15(3), 607-640. <https://doi.org/10.3390/ijpb15030046>
- [9] Hussain, H., & Nisar, M. (2020). Assessment of plant genetic variations using molecular markers: A review. *J. Appl. Biol. Biotechnol*, 8(5), 99-109.
- [10] Tandi, M. R., & Shrirao, N. M. (2025). Optimizing sustainable energy microgrids in smart cities using IoT and renewable energy integration. *Journal of Smart Infrastructure and Environmental Sustainability*, 2(1), 45–50.
- [11] Mas-Ud, M. A., Matin, M. N., Fatamatuzzohora, M., Ahamed, M. S., Chowdhury, M. R., Paul, S. K., ... & Hossain, M. S. (2022). Screening for drought tolerance and diversity analysis of Bangladeshi rice germplasms using morphophysiology and molecular markers. *Biologia*, 77(1), 21-37. <https://doi.org/10.1007/s11756-021-00923-6>